

IMMUNO-DOT BLOT ASSAY

[\(Wright & Morton, 1989\)](#)

Introduction

This assay may be used as a positive or negative test for presence of glomalin but not for determining concentration. It also gives a long-term result, since the color is on the nitrocellulose membrane and does not fade ([Fig. 1](#)).

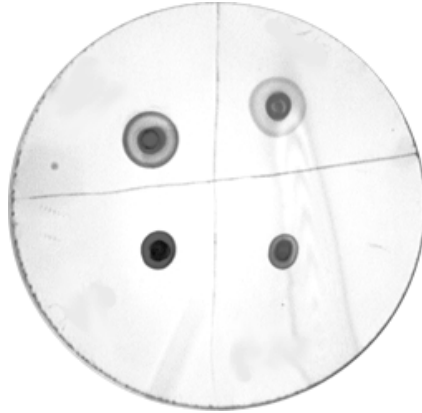


Figure 1. Assay conducted in June, 1998 on glomalin samples from a pot culture experiment.

Materials

0.2 um nitrocellulose membrane

Petri dish or dish to incubate the membrane in

2% non-fat milk (2 g powdered milk/100 ml PBS)

PBS (phosphate buffered saline), pH 7.4

PBST (PBS with Tween 20), pH 7.4

1% BSA* (1 g BSA/100 ml PBS)

TBS (Tris buffered saline) (20mM tris and 250mM NaCl)

MAb32B11 antibody (diluted with PBS, typically 1 ml in 5 ml PBS)**

Biotinylated anti-mouse IgM (4.8 ul/6 ml 1% BSA)**

ExtraAvidin peroxidase (3.0 ul/ 6 ml 1% BSA)**

4-chlor-1-naphthol

methanol

30% hydrogen peroxide

dissecting needle

shaker or tilt table

*Make about 500-1000 ml of stock and dispense in 6 ml aliquots that will be frozen until needed.

**These are recommended concentrations. Chemicals obtained from different companies or with different lot numbers may need to be optimized for your conditions. [Optimize for ELISA](#) and use the same concentrations in this assay.

Methods

- 1) Divide membrane into small squares by drawing lines with the dissecting needle.
- 2) Place 1ul of sample in each square. (Typically the sample is undiluted, but if it is a concentrated sample, you may need to dilute it with PBS.)
- 3) Block membrane with 2% non-fat milk by shaking for 15 min.
- 4) Remove membrane, place on paper towel and dump out solution.
- 5) Add diluted MAb32B11 and incubate on shaker for 1 hr.
- 6) Remove antibody as described in step 4.
- 7) Add PBST, incubate on shaker for 5 min, and discard PBST as described in step 4. Repeat PBST incubation twice.
- 8) Incubate diluted biotinylated anti-mouse IgM with the membrane for 1 hr on shaker.
- 9) Remove IgM (see step 4), add PBST, incubate on shaker for 5 min, and discard PBST. Repeat PBST incubation twice.
- 10) Add diluted ExtrAvidin peroxidase and incubated for 1 hr on shaker.
- 11) Remove peroxidase, add PBST and incubate on shaker for 5 min. Repeat PBST incubation twice and once with TBS.
- 12) Develop with [color developer \(see below\)](#) until color is seen. Remove and dry. Store dry at room temp.

Color developer

- A. Make just prior to using.
- B. Mix 0.015g 4-chlor-1-naphthol in 5ml ice cold methanol.
- C. Immediately before use, add 25 ml TBS plus 15 ul of 30% hydrogen peroxide.